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ORIGINAL ARTICLE

No Evidence for Significant Effect of Body Size and Age on Male Mating Success in the Spot-legged Treefrog

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Abstract In anurans, body size and age of individuals generally affect male mating success. To test whether body size and age have effects on male mating success in the foam-nesting treefrog *Polypedates megacephalus*, a species widely distributed in China, we analyzed differences in body size and age between mated and unmated males for three populations using a Generalized Linear Mixed Model (GLMM). The results showed that mated males did not exhibit larger body size and older age than unmated males, suggesting that large and/or old male individuals did not have greater mating success than small and/or young males. Moreover, we also found a non-significant size-assortative mating pattern for all populations. Our findings suggest that body size and age of the foam-nesting treefrog do not affect male mating success.

Keywords age, body size, male mating success, *Polypedates megacephalus*, size-assortative

1. Introduction

Sexual selection theory suggests that parental reproductive investment determines which sex is choosy with respect to mate choice and which sex contests (Darwin, 1871). A majority of studies on sexual selection have shown that females are generally the choosier sex and that males compete for mates (Emlen and Oring, 1977; Liao and Lu, 2009a; Sih *et al*., 2014). As a result, sexual selection can promote male mating success and/or female reproductive success (Andersson, 1994).

In amphibians, numerous studies on sexual selection reveal that relatively high values of traits in males (i.e. body size, forearm length and thumb-pad width) will be preferred by females (Bell, 2010; Liao and Lu 2011a, b). As the most important trait, body size is linked to the males' physical strength and the ability to compete for females (Halliday, 1983). Size-dependent mating success, in which large males have higher mating opportunity than small males, is generally interpreted as the consequence of male-male competition (Cothran, 2008; Chen and Lu, 2011; Liao and Lu, 2011b). This is a general pattern in the animal kingdom because large males exhibit more dominance than small males when they compete for access to females or maintain resources necessary for females (Clutton-Brock *et al*., 1992; Awata *et al*., 2006; Liao and Lu, 2011b). Moreover, body size also serves as a basis for female mate choice not only because large males may be older and consequently have demonstrated their ability to survive (Halliday, 1983; Wilbur *et al*., 1978; Wellborn and Cothran, 2007; Liao and Lu, 2011a), but also because females may gain indirect benefits from being choosy by passing on good genes to offspring or sons of large males (Andersson, 1994). In some anuran species, male mating success is also correlated with age because old individuals have better survival rate and more successful egg fertilization than do young ones (Byrne and Whiting, 2008; Liao and Lu, 2011a; Somashekar and Krishna, 2011; Kierl and Johnston, 2015).

The spot-legged treefrog (*Polypedates megacephalus*) is widely distributed in China, at altitudes ranging from 520 m to 2200 m (Fei and Ye, 2001). Breeding activity ranges from mid-April to late July. It is a lekking species where males gather at ponds to wait for females. Females are only present at these ponds during the night for

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mating. Amplectant pairs gather at the edge of the pond and then release foam above the pond's water surface. Immediately, other males came to join the pair, forming an amplexing group of 2 to 5 males. There is no evidence for scramble competition, combats, pushing etc. between males in nests. Except for the knowledge presented above, there is no detailed information about the effect of body size and age on male mating success in the species. The present study is a combination of field collection and skeletochronology to analyze the effect of both body size and age on male mating success in the spot-legged treefrog. More specifically, our aims were (1): to analyze the correlation of body size between amplectant males and amplectant females to confirm size-assortative mating pattern for all populations; (2) to compare the body size between mated and unmated males to gain insight into the relationship between male mating success and body size; (3) to test the relationship between male mating success and age by comparing the age between mated and unmated males.

2. Material and Methods

2.1 Study area We collected three *P. megacephalus* populations located at different altitudes in Guizhou province in western China during the successive breeding seasons (2014 and 2015). The low-altitude site was located at 449 m in Fanjing mountains (108°44.67' E, 27°46.33' N) where the treefrogs reproduce in a farm (5.0 × 3.6 × 2.8 m^3; L × W × H) surrounded by farmhouse. The middle-altitude site was located at 680 m in Shangzhong town (108°43.38' E, 27°23.38' N) where the treefrogs reproduce in a farm (3.0 × 4.2 × 3.8 m^3; L × W × H) near a small pigfarm. The high-altitude site was located at 1300 m in Leigong mountains (108°10.27' E, 26°22.73' N) where the treefrogs reproduce in two natural ponds (2.0 × 1.5 × 1.2 m^3, 12.0 × 4.0 × 0.3 m^3; L × W × H) surrounded by farmhouse.

2.2 Samplings We conducted field observations for all populations during the breeding season in 2014 and 2015. We recorded the amplectant males and unamplectant males in the pond. The amplectant and unamplectant males were distinguished by the condition where males attend to the group spawning. Within each breeding site we captured all males and females that spend the nights at the pond after laying eggs of all females. For the three breeding sites, the females produce eggs exceeding twenty days. We collected a total of 209 tree frogs (Leigong: 58 males and 20 females; Shangzhong: 48 males and 21 females; Fanjing: 41 males and 21 females). We confirmed all individuals as adults though observation of secondary sexual traits (presence of nuptial thumb pads in adult males and the inflated abdomen carrying the developing eggs of adult females). We measured body size (snout-vent length: SVL in mm) of each individual to the nearest 0.1 mm using a vernier calipers. The second phalange of the longest hind finger from the right hindlimb of each treefrog was removed and stored in 4% neutral buffered formalin for age determination (see below). Each treefrog was released at the capture site.

2.3 Age determination We used skeletochronology which counts the number of lines of arrested growth (LAGs) in stained cross sections of the phalangeal bones to determine age of each individual (see Castanet and Smirina, 1990). An improved method of paraffin sectioning and Harris's haematoxylin staining to produce histological sections was used for aging adult females and males. The method has been successfully used in many Chinese anurans (Liao and Lu, 2010; Liao and Lu, 2012; Li *et al*., 2013; Huang *et al*., 2013). Cross-sections (13 μm thick) of the phalanx with the smallest medullar cavity was used to mount on glass slides. Then we recorded the number of lines of LAGs, taken from mid-diaphyseal sections, using a Motic BA300 digital camera mounted on a Moticam2006 light microscope at×400 magnification. We also analyzed LAG endosteal resorption and double lines following the methods of Castanet and Smirina (1990). Of 209 adult specimens, 207 (145 males and 62 females) exhibited clear LAGs in their bone sections.

2.4 Data analysis All analyses were performed by using the SPSS 21.0 statistical package. Body size was $\log_{10}$-transformed. To assess size-assortative mating pattern for the three populations, we analyzed the correlation of body size between males and females using a Pearson correlation analysis. To test differences in mean body size between paired and unpaired males across altitudes, we used a Generalized Linear Mixed Model (GLMM) with $\log_{10}$ (SVL) or age as the dependent variable, mating status as a fixed factor, and population as a random effect, altitude as covariate. We ran a GLMM with age and altitude as covariate to test differences in body size of paired and unpaired males after removing the effects of age.

3. Results

The breeding season differed significantly across the three breeding sites (Leigong: 18–30 April; Fanjing: 1–12 May; Shangzhong: 14–24 May). Reproduction lasted

four weeks. Sexual activity started at sunset (20:00), with males usually arriving at the breeding sites first, and finished when the last males departed (02:30–03:30). The whole process of oviposition lasted 30–80 minutes. We gathered 124 treefrogs (62 pairs) in amplexus. Females had significantly larger body size than males (Table 1). Comparison of SVL of mated pairs revealed a non-significant size-assortative mating patterns by body size for the three populations (Figure 1; Leigong: $r = -0.024$, $n = 20$, $P = 0.918$; Shangzhong: $r = -0.073$, $n = 21$, $P = 0.752$; Fanjing: $r = 0.274$, $n = 21$, $P = 0.229$).

The GLMM revealed that there was also non-significant difference in average body size between mated and unmated males ($F_{1, 0.435} = 0.667$, $P = 0.669$; Table 1) and among altitudes across populations (altitude: $F_{1, 4.598} = 1.375$, $P = 0.298$; population: $Z = 0.207$, $P = 0.836$). Likewise, average age did not differ between mated and unmated males (GLMM: $F_{1, 142} = 0.409$, $P = 0.523$; Table 1) and among altitudes ($F_{2, 142} = 1.843$, $P = 0.177$). There was also non-significant difference in body size between mated and unmated males (GLMM: $F_{1, 32.269} = 0.698$, $P = 0.410$) when controlling the effects of age and altitude on body size (age: $F_{1, 138.686} = 23.400$, $P < 0.001$; altitude: $F_{1, 32.269} = 0.698$, $P = 0.410$, $P = 0.121$; population: $Z = 0.667$, $P = 0.505$).

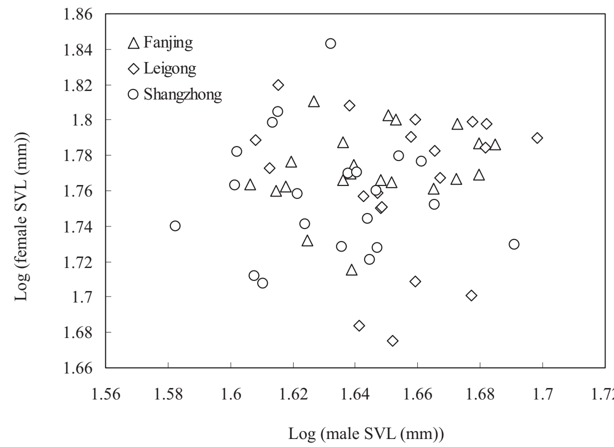

Figure 1 A non-significant size-assortative mating pattern in *Polypedates megacephalus* for the three populations.

4. Discussion

Our results uncover a non-significant size-assortative mating patterns all *P. megacephalus* populations. Furthermore, mean body size and age do not differ between mated and unmated males in *P. megacephalus*, suggesting that large or old males do not exhibit higher mating success than do small or young males. Size-assortative mating pattern can increase fertilization rate by cloacal apposition during spawning (Halliday, 1983). Previous studies have indicated that size-assortative mating in several anurans improves the apposition of female and male cloacae while spawning (*Bufo bufo*, Davies and Halliday, 1977; *Triprion petasatus*, Lee and Crump, 1981; *Hyla elegans*, *Hyla labialis*, Gutiérrez and Lüddecke, 2002; *Agalychnis callidryas* and *A. moreletii*, Briggs, 2008; *B. andrewsi*, Liao and Lu, 2012). For the treefrog, fertilization rate was not improved by the apposition of female and male cloacae, but group spawning. Moreover, size-assortative mating pattern may be the outcome of competition among males for females (Arak, 1983). However, we did not find a positive correlation of body size between mated males and mated females for 62 pairs. This pattern may attribute to weak male-male competition for females in *P. megacephalus* when 2-5 males gather at foam nests.

Difference in body size was non-significant between mated and unmated males in *P. megacephalus* even when controlling for the effect of age and altitude which suggests that large males did not exhibit higher opportunity to obtain mates. This is inconsistent with the prediction that sexual selection act on body size in the related foam-nest species due to female mate choice depending on male calls: larger males have higher call

Table 1 Means and standard deviations of body size, age and sampling for three populations of *Polypedates megacephalus*.

Characters	Body size (mm)			Age (years)		
	Females	Paired males	Unpaired males	Females	Paired males	Unpaired males
Leigong	58.4 ± 5.4	45.1 ± 2.5	42.8 ± 4.7	3.0 ± 0.9	3.0 ± 0.8	2.9 ± 0.8
	n = 20	*n* = 20	*n* = 38	*n* = 20	*n* = 20	*n* = 38
Shangzhong	57.4 ± 4.5	43.0 ± 2.5	43.0 ± 3.1	2.9 ± 0.5	2.9 ± 0.9	2.9 ± 0.8
	n = 21	*n* = 21	*n* = 28	*n* = 21	*n* =21	*n* = 28
Fanjing	59.3 ± 3.0	44.3 ± 2.4	43.2 ± 3.1	2.8 ± 0.8	2.6 ± 0.7	3.0 ± 0.8
	n = 21	*n* = 21	*n* = 20	*n* = 21	*n* =21	*n* = 18

frequencies, and females can exploit this information (i.e. *R. schlegelii*, Fukuyama, 1991; *R. arboreus*, Kusano *et al*., 1991; *R. omeimontis*, Liao and Lu, 2011a). For most anurans, body size cannot be a good indicator of male competing advantages, thus, large body size did not reflect a male's ability to compete for a female (Davies and Halliday, 1977; Gutiérrez and Lüddecke, 2002; Liao and Lu, 2009b; Liao *et al*., 2015). In this study, body size cannot be a good indicator of male competing advantages because intensive sperm competition in group spawning trade off male-male competition. Similar results have been observed in other frogs and toads (Halliday 1983; Liao and Lu, 2011a).

Male attributes that it is important for consideration in females include controlling resources and genetic quality (Howard, 1978; Sih *et al*., 2014). For anurans, strong competitive ability in old males shows a high genetic quality (Wilbur *et al*., 1978; Trivers, 1972). In the study, we did not find difference in mean age between mated and unmated males in *P. megacephalus,* suggesting that old males did not exhibit higher mating success. This is inconsistent with the hypothesis in frogs that females mating with old males would gain indirect genetic benefits in terms of improved offspring fitness (Wilbur *et al*., 1978; Howard, 1984; Liao and Lu, 2011a).

Age shows a positive correlation with male advertisement calls with a high rate or long duration, thus, female preference for males with high rate or long duration equates with choosing old males (Welch *et al*., 1998; Liao and Lu, 2011a). In the study, we did not find females preference for old males, suggesting that there may not be indirect genetic benefits for females choosing males with long calls. Hence, further investigation is warranted to analyze the relationships between male call rates, female preferences and male age.

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